Removal of the Kanamycin resistance gene in *E. coli* strain JW0336 by FLP recombination in *Escherichia coli*

1. Chemical competent cells of *E. coli* JW0336 were prepared with TSS method.

Day 1: Transformation of the recombinase plasmid

1. The competent cells were transformed with plasmid pCP20, which had a temperature-sensitive origin of replication resistant to ampicillin and chloramphenicol. With the presence of the FLP recombinase, the ampicillin and chloramphenicol resistance genes would be removed under the temperature higher than 43°C.
2. 1 ml LB was added for recovery which lasted for 1 hour.
3. The eppendorf was centrifuged for 2.5 minutes with 4 krpm. Supernatant was discarded and the pellet was resuspended and plated on the LB + AMP plate.
4. The plate was then incubated for 1.5 days under 30°C.

Day 2: Induction of the recombination

1. Three colonies were picked and inoculated into 5 ml LB in Falcon tube.
2. The tubes were shaken overnight at 45°C so as to induce FLP recombinase expression and select for the loss of pCP20.

Day 3: Plating to get single candidate recombinants

1. 100-fold dilution of the overnight culture was made by using fresh LB.
2. 50 ul of the diluted overnight culture was plated on LB plate.
3. The plate was then incubated for 1.5 days under 30°C to prevent partial loss of plasmid from the colonies which developed from the cells containing pCP20.

Day 4: Screening for genomic recombination and plasmid loss

1. 6 colonies were picked from each plates and resuspended in 0.85% NaCl.
2. Little amount of the 0.85% NaCl with cells was streaked on 4 different types of plates: LB, LB + AMP, LB + CHL and LB + KAN for screening.
3. The plate was then incubated overnight under 37°C.

Day 5: Obtaining successful recombinants

1. The candidate which was sensitive to all antibiotics would be selected and archived.

References

Datsenko, K.A., and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proc. Natl. Acad. Sci. U.S.A.* **97**: 6640-6645.

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